



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b>  A61K 49/02	<b>A1</b>	<b>(11) International Publication Number:</b> WO 92/13572 <b>(43) International Publication Date:</b> 20 August 1992 (20.08.92)
<b>(21) International Application Number:</b> PCT/US92/00757 <b>(22) International Filing Date:</b> 7 February 1992 (07.02.92) <b>(30) Priority data:</b> 653,012                      8 February 1991 (08.02.91)      US  <b>(71) Applicant:</b> DIATECH, INC. [US/US]; 9 Delta Drive, Londonderry, NH 03053 (US). <b>(72) Inventor:</b> DEAN, Richard, T. ; 43 King Road, Bedford, NH 03102 (US). <b>(74) Agent:</b> NOONAN, Kevin, E.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).		<b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> TECHNETIUM-99m LABELED POLYPEPTIDES FOR IMAGING  <b>(57) Abstract</b>  The invention relates to radiolabeled imaging of a mammalian body. The invention in particular provides for reagents labeled with technetium-99m for such imaging. The invention provides peptides which bind technetium-99m and which can be targeted to specific sites within a mammalian body.		

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## TECHNETIUM-99m LABELED POLYPEPTIDES FOR IMAGING

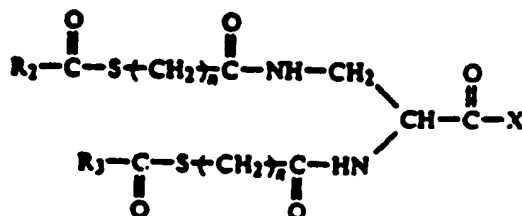
### BACKGROUND OF THE INVENTION

## Field of the Invention

5 This invention relates to radiodiagnostic reagents and, more particularly, to polypeptides useful for producing technetium (Tc-99m) labeled radiodiagnostic agents. The invention relates to Tc-99m labeled reagents, kits for making such reagents, and methods for using such reagents.

### Description of the Prior Art

10 U.S. Patent No. 4,861,869 (Nicolotti) describes coupling agents of the formula:

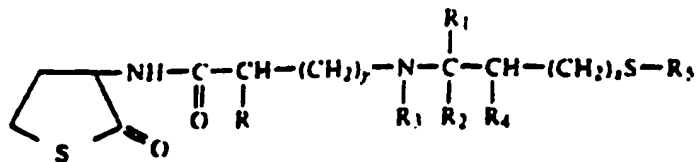


wherein  $R_2$  and  $R_3$  are the same or different and each represents a radical selected from the group consisting of alkyls having from 1 to 6 carbon atoms, aryls having from 6 to 8 carbon atoms and alkylaryls having 7 to 9 carbon atoms, any of which can be substituted with one or more hydroxyl, alkoxy, carboxy or sulfonate groups;  $n$  is either 1 or 2; and  $X$  is an activating group capable of forming an amide bond with an alpha or beta amino group of a biologically useful protein or polypeptide molecule.

U.S. Patent No. 4,861,869 also describes compounds such as S-  
20 benzoylmercaptoacetylglycylglycylglycine.

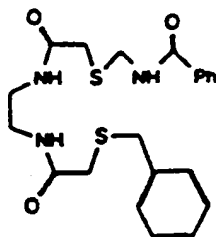
The coupling agents are bound to large peptides such as antibodies or fragments thereof and complexed to Tc-99m.

U.S. Patent Nos. 4,571,430, 4,575,556 and 4,434,151 (Byrne et al.) describe compounds of the formula:



wherein R is hydrogen or lower alkyl, R<sub>1</sub> and R<sub>2</sub> are individually hydrogen or lower alkyl or taken together form oxo; R<sub>3</sub> is an amino protecting group where R<sub>1</sub> and R<sub>2</sub> taken together form oxo; R<sub>4</sub> is hydrogen or lower alkyl; R<sub>5</sub> is hydrogen or a thiol protecting group; and y and z are integers from 0 to 2; which are bifunctional chelating agents and as such can couple radionuclides to terminal amino-containing compounds capable of localizing in an organ or tissue which is desired to be imaged.

Bryson et al., *Inorg. Chem.* 27: 2154-2161 (1988) and *Inorg. Chem.* 29: 2948-2951 (1990), describes thiolate ligands for complexing with technetium of the formula:



European Patent Application No. 86100360.6, filed January 13, 1986, describes dithio, diamino, or diamidocarboxylic acids or amine complexes useful for making technetium imaging agents.

Other references of interest include Khaw et al., *J. Nucl. Med.* 23: 1011 (1982); Rhodes, B.A., *Sem. Nucl. Med.* 4: 281 (1974); Davidson et al., *Inorg. Chem.* 20: 1629 (1981); and Byrne and Tolman, *J. Nucl. Med.*

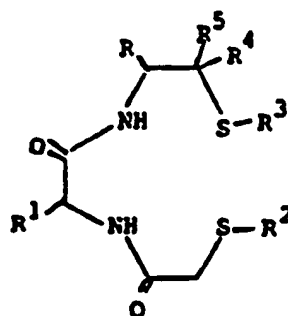
24: 126 (1983). See particularly Fritzberg et al., *J. Nucl. Med.* 23: 592 (1982); Fritzberg et al., *ibid.* 23: 17 (1982), for descriptions of mercaptoacetyl derivatives of ethylene diamine carboxylic acid derivatives. See also U.S. Patent Nos. 4,434,151, 4,444,690 and 4,472,509.

5 European Patent Application 88104755.9 describes various S-protected mercaptoacetylglycylglycine chelating groups bound to large proteins such as antibodies.

European Patent Application 84109831.2 describes technetium complexes of compounds of the formula I and II:

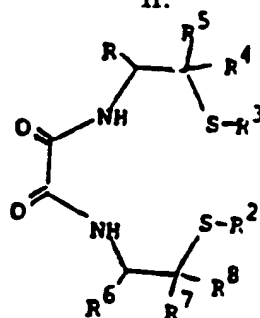
10

I.



and

II.



15

wherein R and R<sub>6</sub> are each selected from hydrogen, substituted or unsubstituted lower alkyl or -COR where R<sub>9</sub> is selected from hydroxy, substituted or unsubstituted lower alkoxy, substituted or unsubstituted amino, glycine ester, or an activated leaving group; R<sub>1</sub> is selected from hydrogen, or substituted or unsubstituted lower alkyl; R<sub>2</sub> and R<sub>3</sub> are each selected from hydrogen or a thiol protecting group; and R<sub>4</sub>, R<sub>5</sub>, R<sub>7</sub>, and R<sub>8</sub> are each selected from hydrogen or lower alkyl; and salts thereof. These complexes

were used primarily as renal function monitoring agents.

Arginylglycylaspartate (Arg-Gly-Asp or RGD) and derivative peptides are known to bind to blood clots (see U.S. Patent Nos. 4,792,525, 4,857,508 and 4,578,079) and RGD derivatives have been labeled with technetium as imaging agents, *Journal of Nuclear Medicine* 31, pp. 757, No. 209 (1990).

### SUMMARY OF THE INVENTION

The invention encompasses polypeptides for labeling with technetium-99m and imaging target sites within a mammalian body comprising (a) a specific binding polypeptide region which specifically binds to the target site to be imaged, and (b) a technetium binding region of the formula Cp(aa)Cp wherein Cp is a protected cysteine and (aa) is an amino acid and wherein the technetium binding region is covalently bound to the specific binding polypeptide region. The invention includes technetium-99m complexes and methods for using the technetium-99m complexes to image target sites within a mammalian body.

### DETAILED DESCRIPTION OF THE INVENTION

The Cp(aa)Cp technetium binding group is covalently linked to the specific binding polypeptide preferably by one or more amino acids, most preferably glycine. Alternatively, the Cp(aa)Cp technetium binding group may be directly covalently linked to the specific binding polypeptide or other covalent linking groups can be used such as bifunctional amino/carboxy compounds which are not naturally-occurring amino acids.

Representative specific binding polypeptide sequences are:

#### Atherosclerotic Plaque Binding Peptides

YRALVDTLK  
RALVDTLK  
RALVDTLKFVTQAEGAK  
YAKFRETLEDTRDRMY  
AKFRETLEDTRDRMY  
YAALDLNAVANKIADFEL

5

Atherosclerotic Plaque Binding Peptides (cont'd.)

5 AALDLNAVANKIADFEL  
 YRALVDTLKFVTEQAKGA  
 RALVDTLKFVTEQAKGA  
 YRALVDTEFKVKQEAGAK  
 RALVDTEFKVKQEAGAK  
 YRALVDTLKFVTQAEGAK

Peptides Targeted to Infections and  
Atherosclerotic Plaque

10 VGVAPGVGVAPGVGVAPG  
 VPGVGVPGVGVPGVGVPGVG  
 formyl.Nleu.LF.Nleu.YK  
 formyl MIFL  
 formyl MLFK  
 15 formyl MLFI  
 formyl MFIL  
 formyl MFLI  
 formyl MLIF  
 formyl MILF  
 20 TKPR  
 VGVAPG  
 formyl MLF

Thrombus

25 NDGDFEEIPEEYLQ  
 NDGDFEEIPEEY(SO<sub>3</sub>Na)LQ  
 GPRG

Platelets

30 D-Phe.PRPGGGGNGDFEEIPEEYL  
 RRRRRRRRRGDV  
 PLYKKIIKKLLES  
 RGD  
 RGDS

Infection and Atherosclerotic Plaque

35 YIGSR  
 CH<sub>2</sub>CO.YIGSRC

Alzheimers Disease (Amyloid Plaque)

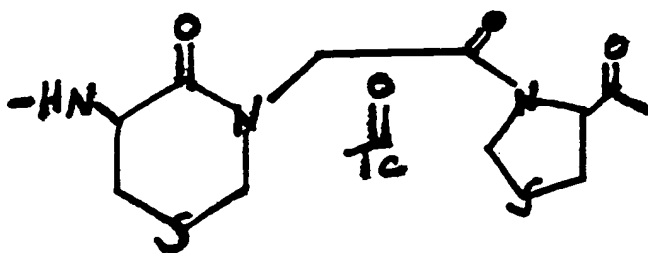
EKPLQNFTLSFR

[Single letter abbreviations for amino acids can be found in G. Zubay, Biochemistry (2d ed.), 1988, (MacMillan Publishing: New York), p. 33.]

5 In the Cp(aa)Cp, the Cp is a protected cysteine where the S-protecting groups are the same or different and may be but not limited to:

- CH<sub>2</sub>-aryl (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- CH-(aryl)<sub>2</sub>, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- C-(aryl)<sub>3</sub>, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- 10 -CH<sub>2</sub>-(4-methoxyphenyl);
- CH-(4-pyridyl)(phenyl)<sub>2</sub>;
- C(CH<sub>3</sub>)<sub>3</sub>;
- 9-phenylfluorenyl;
- CH<sub>2</sub>NHCOR (R is unsubstituted or substituted alkyl or aryl);
- 15 -CH<sub>2</sub>-NHCOOR (R is unsubstituted or substituted alkyl or aryl);
- CONHR (R is unsubstituted or substituted alkyl or aryl);
- CH<sub>2</sub>-S-CH<sub>2</sub>-phenyl

When Cp-gly-Cp is combined with technetium, the following complex with the protecting groups removed is formed:



20 The preferred protecting group has the formula -CH<sub>2</sub>-NHCOR wherein R is a lower alkyl having 1 and 8 carbon atoms, phenyl or phenyl-substituted with lower alkyl, hydroxyl, lower alkoxy, carboxy, or lower alkoxy carbonyl.

Compounds of the present invention can generally advantageously be



prepared on an amino acid synthesizer. Compounds of this invention are advantageous in that they are soluble and the sulfur is stabilized.

5 In forming the complex of radioactive technetium with the compounds of this invention, the technetium complex, a salt of technetium-  
99m pertechnetate, is reacted with the compound of this invention in the  
presence of a reducing agent such as stannous chloride ferrous ion or  
sodium dithionite. These technetium labeled complexes can also be made  
by exchange of a prereduced technetium -99m complex. The complexes are  
conveniently provided in a kit form comprising a sealed vial containing a  
10 predetermined quantity of a compound to be labeled and a sufficient amount  
of reducing agent to label the compound with technetium-99m.  
Alternatively, the complex may be formed by reacting the compound of this  
invention with a pre-formed labile complex of technetium and another  
compound. This process is known as ligand exchange, is well known to  
15 those skilled in the art, and the labile complex may be formed using such  
compounds as tartrate, citrate, gluconate or mannitol, for example. Among  
the technetium-99m pertechnetate salts are included the alkali metal salts  
such as the sodium salt or ammonium salts, or lower alkyl ammonium  
salts. The reaction of the compound of this invention with pertechnetate  
20 or preformed labile complex can be carried out in an aqueous medium at  
room temperature. The anionic complex which has a charge of -1 is  
formed in the aqueous medium in the form of a salt with a suitable cation  
such as sodium, ammonium cation, mono, di- or tri-lower alkyl amine  
cation, etc. Any conventional salt of the anionic complex with a  
25 pharmaceutically acceptable cation can be used in accordance with this  
invention.

In carrying out the reaction of the compounds of this invention with  
pertechnetate or a labile complex to form the anionic complex, the thiol  
protecting group is cleaved. Therefore, this reaction not only introduces  
30 the radioactive metal into the compound but also cleaves the thiol protecting  
group. All of the aforementioned thiol protecting groups are cleaved by a

reaction of salts of radioactive metals in accordance with this invention.

5 In forming the complex the radioactive material has a suitable amount of radioactivity. In forming the Tc-99m radioactive anionic complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 milliCuries (mCi) to 100 mCi per ml.

10 The complex can be used for visualizing organs such as the kidney for diagnosing disorders in these organs, tumors and blood clots can also be imaged. In accordance with this invention, the anionic complex either as a complex or as a salt with a pharmaceutically acceptable cation is administered in a single unit injectable dose. Any of the common carriers such as sterile saline solution, plasma, etc., can be utilized after the radiolabeling for preparing the injectable solution to diagnostically image various organs, clots, tumors and the like in accordance with this invention.

15 Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 ml to about 10 ml. After intravenous administration, imaging of the organ *in vivo* can take place in a matter of a few minutes. However, imaging can take place, if desired,

20 in hours or even longer, after injecting into patients. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of imaging for diagnostic purposes can be utilized in accordance with this invention.

25 The complexes may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Such medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Among the preferred mediums are normal saline

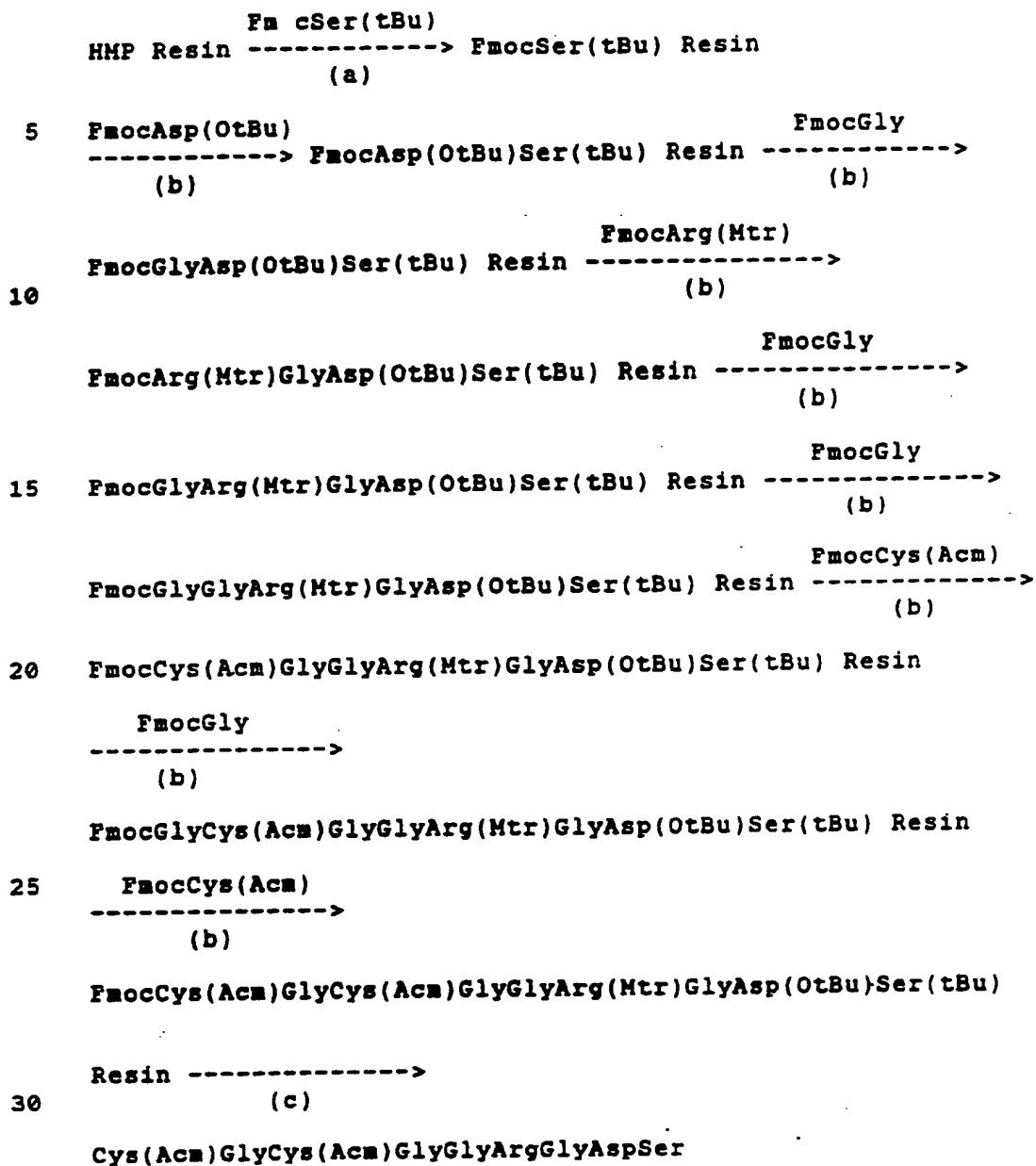
30 and plasma.

The methods for making and labeling these compounds are more fully illustrated in the following examples.

Example 1

Cys(Acm)GlyCys(Acm)GlyGlyArgGlyAspSer

5           The title compound was prepared on a 0.25 millimole scale using  
an Applied Biosystems Model 431A peptide Synthesizer, N-terminus Fmoc  
protection and HMP resin (see Scheme). The product was cleaved from  
the resin using 95% trifluoroacetic acid at room temperature for 3 hours.  
Work-up and high performance liquid chromatography (HPLC) purification  
10       (using a Vydac 2.20cm x 25cm, 10um, C-18 column with a 20-minute  
gradient of 0.1% trifluoroacetic acid to 70% acetonitrile/ 0.1%  
trifluoroacetic acid at a flow rate of 25 ml/min) gave 50 mg of the title  
compound, 95% pure. (HPLC peak eluted at 5.5 min; Pos. ion FABMS  
Calc MM 952.97, Found 953).

Scheme for Preparation of the Title Compound

- (a) DCC, HOB, NMP
- (b) 1.piperidine, NMP, 2. DCC, HOB, NMP
- (c) 1.piperidine, NMP, 2. 95% CF<sub>3</sub>CO<sub>2</sub>H, 3. HPLC
- 35 DCC = dicyclohexylcarbodiimide
- HOB = hydroxybenztriazole
- NMP = N-methylpyrrolidinone
- HMP = p-hydroxymethylphenoxymethylpolystyrene
- Fmoc = 9-fluorenylmethoxycarbonyl
- 40 tBu = tert-butyl
- Mtr = 4-methoxy-2,3,6-trimethylbenzenesulfonyl
- Acm = acetamidomethyl

Example 2Radiolabeling of Compound of Example 1 with Tc-99m

0.3 mg of the compound prepared as in Example 1 was dissolved in 0.3 ml of 0.05M potassium phosphate buffer (pH 7.4) containing 0.5 mM EDTA. Tc-99m gluceptate was prepared by reconstituting a Glucoscan vial (E.I. DuPont de Nemours, Inc.) with 1.0 ml of Tc-99m sodium pertechnetate containing 26 mCi. After 15 minutes at room temperature, 75 ul of Tc-99m gluceptate was added to 0.3 mg of the compound prepared as in Example 1 and boiled for 45 minutes.

The extent of Tc-99m labeling of the peptide was determined by chromatography using Merck silica gel 60 F<sub>250</sub> aluminum-backed strips which were spotted with 10 ul of sample and chromatographed with acetonitrile:0.5M sodium chloride solvent (15:85) approximately 2% of Tc-99m radioactivity remained at R<sub>f</sub> 0.0, confirming that no significant Tc-99m colloids or aggregates were generated.

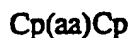
The Tc-99m labeled peptide purity was determined by HPLC using a Brownlee Spheri-5 (5um) resin, RP-18, 220 x 4.6 mm column and the following gradient: 0% A (CH<sub>3</sub>CN:H<sub>2</sub>O:TFA, 70:30:0.1) and 100% B (0.1% TFA in H<sub>2</sub>O) to 100% A + 0% B over 10 minutes at 1.5 ml/min; and then held at the 100% A solvent for 5 minutes. This protocol yielded 100% of the radiometric species detected (by in-line NaI detector) as a single species (retention time = 10.9 min). Tc-99m gluceptate and Tc-99m sodium pertechnetate elute between 1 and 4 minutes under identical conditions, confirming the identity of the Tc-99m labeled peptide isolated.

What is claimed is:

1. A polypeptide for labeling with technetium-99m and imaging target sites within a mammalian body comprising:

(a) a specific binding polypeptide region which specifically binds to the target site to be imaged and

(b) a technetium binding region of the formula



wherein Cp is a protected cysteine and (aa) is an amino acid and wherein the technetium binding region is covalently bound to the specific binding polypeptide region.

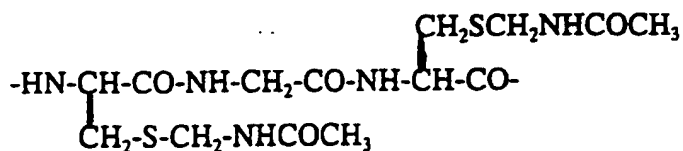
2. A polypeptide according to claim 1 wherein the specific binding polypeptide region and Cp(aa)Cp is covalently linked through one or more amino acids.

3. A polypeptide according to claim 1 wherein the protected cysteine has a protecting group of the formula



wherein R is a lower alkyl having 1 to 6 carbon atoms, phenyl, or phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower alkoxycarbonyl, or 2-,3-,4-pyridyl.

4. A polypeptide according to claim 1 wherein Cp(aa)Cp has the formula:



5. A polypeptide according to claim 1 wherein the specific

binding polypeptide region specifically binds to clots, tumors, sites of infection, atherosclerotic plaques, amyloid plaques or bone.

- 5 6. A polypeptide according to claim 1 wherein the specific binding polypeptide region is selected from polypeptides consisting of the amino acid sequences:

YRALVDTLK  
RALVDTLK  
RALVDTLK FVTQAEGAK  
YAKFRETLEDTRDRMY  
10 AKFRETLEDTRDRMY  
YAALDLNAVANKIADFEL  
AALDLNAVANKIADFEL  
YRALVDTLK FVTEQAKGA  
RALVDTLK FVTEQAKGA  
15 YRALVDTEFKVKQEAGAK  
RALVDTEFKVKQEAGAK  
YRALVDTLK FVTQAEGAK  
VGVAPGVGVAPGVGVAPG  
VPGVGVPGVGVPGVGVPGVG  
20 formyl.Nleu.LF.Nleu.YK  
formyl MIFL  
formyl MLFK  
formyl MLFI  
formyl MFIL  
25 formyl MFLI  
formyl MLIF  
formyl MILF  
TKPR  
VGVAPG  
30 formyl MLF  
NDGDFEEIPEEYLQ  
NDGDFEEIPEEY(SO<sub>3</sub>Na)LQ  
GPRG  
D-Phe.PRPGGGGNGDFEEIPEEYL  
35 RRRRRRRRRGDV  
PLYKKIKKLLS  
RGD  
RGDS  
YIGSR  
40 CH<sub>3</sub>CO.YIGSRC  
EKPLQNFTLSFR

7. The polypeptide of claim 6 bound to technetium-99m.

8. A complex formed by reacting a compound of claim 1 with technetium-99m in the presence of a reducing agent.

5 9. The complex of claim 8, wherein the said reducing agent is selected from the group of a dithionite ion, a stannous ion, or a ferrous ion.

10 10. A complex formed by labelling a compound of claim 1 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.

11. A kit for preparing a radiopharmaceutical preparation, said kit comprising sealed vial containing a predetermined quantity of a compound of claim 1 and a sufficient amount of reducing agent to label said compound with technetium-99m.

15 12. A method for imaging a target site within a mammalian body comprising administering an effective diagnostic amount of a polypeptide of claim 1 which is labeled with technetium-99m and wherein the specific binding polypeptide region binds to the target site, and detecting the localized technetium-99m.

20 13. The process of preparing the peptide according to Claim 1 wherein the peptide is chemically synthesized *in vitro*.

14. The process of preparing the peptide according to Claim 13 wherein the peptide is synthesized by solid phase peptide synthesis.



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 92/00757

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC Int.C1.5                      A 61 K 49/02																				
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border: 1px solid black; padding: 2px;">Minimum Documentation Searched<sup>7</sup></div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 25%; padding: 5px;">Classification System</td> <td style="padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">Int.C1.5</td> <td style="padding: 5px;">A 61 K</td> </tr> </table> <div style="text-align: center; padding: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched<sup>8</sup></div>			Classification System	Classification Symbols	Int.C1.5	A 61 K														
Classification System	Classification Symbols																			
Int.C1.5	A 61 K																			
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category<sup>o</sup></th> <th style="width: 70%; padding: 5px;">Citation of Document,<sup>11</sup> with indication, where appropriate, of the relevant passages<sup>12</sup></th> <th style="width: 20%; padding: 5px;">Relevant to Claim No.<sup>13</sup></th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">WO,A,9015818 (ANTISOMA LTD) 27 December 1990, see the whole document ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">WO,A,9006323 (CENTOCOR INC.) 14 June 1990, see page 2, line 22 - page 4, line 6; page 8, lines 9-17; page 17, example 2; claims ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP,A,0284071 (NEORX) 28 September 1988, see the whole document (cited in the application) ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP,A,0137457 (E.I. DU PONT DE NEMOURS AND CO.) 17 April 1985, see the whole document ---</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-14</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP,A,0055028 (MINNESOTA MINING AND MANUFACTURING CO.) 30 June 1982, see the whole document, especially page 16, lines 22-35 --- -/-</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-14</td> </tr> </tbody> </table>			Category <sup>o</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	Y	WO,A,9015818 (ANTISOMA LTD) 27 December 1990, see the whole document ---	1-14	Y	WO,A,9006323 (CENTOCOR INC.) 14 June 1990, see page 2, line 22 - page 4, line 6; page 8, lines 9-17; page 17, example 2; claims ---	1-14	Y	EP,A,0284071 (NEORX) 28 September 1988, see the whole document (cited in the application) ---	1-14	Y	EP,A,0137457 (E.I. DU PONT DE NEMOURS AND CO.) 17 April 1985, see the whole document ---	1-14	Y	EP,A,0055028 (MINNESOTA MINING AND MANUFACTURING CO.) 30 June 1982, see the whole document, especially page 16, lines 22-35 --- -/-	1-14
Category <sup>o</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>																		
Y	WO,A,9015818 (ANTISOMA LTD) 27 December 1990, see the whole document ---	1-14																		
Y	WO,A,9006323 (CENTOCOR INC.) 14 June 1990, see page 2, line 22 - page 4, line 6; page 8, lines 9-17; page 17, example 2; claims ---	1-14																		
Y	EP,A,0284071 (NEORX) 28 September 1988, see the whole document (cited in the application) ---	1-14																		
Y	EP,A,0137457 (E.I. DU PONT DE NEMOURS AND CO.) 17 April 1985, see the whole document ---	1-14																		
Y	EP,A,0055028 (MINNESOTA MINING AND MANUFACTURING CO.) 30 June 1982, see the whole document, especially page 16, lines 22-35 --- -/-	1-14																		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>o</sup> Special categories of cited documents :<sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>																				
<b>IV. CERTIFICATION</b> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;">           Date of the Actual Completion of the International Search   <div style="text-align: center;">09-06-1992</div> </td> <td style="width: 50%; padding: 5px;">           Date of Mailing of this International Search Report   <div style="text-align: center;">29. 07. 92</div> </td> </tr> <tr> <td style="width: 50%; padding: 5px;">           International Searching Authority   <div style="text-align: center;">EUROPEAN PATENT OFFICE</div> </td> <td style="width: 50%; padding: 5px;">           Signature of Authorized Officer  </td> </tr> </table>			Date of the Actual Completion of the International Search  <div style="text-align: center;">09-06-1992</div>	Date of Mailing of this International Search Report  <div style="text-align: center;">29. 07. 92</div>	International Searching Authority  <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer 														
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International Searching Authority  <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer 																			

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	WO,A,9010463 (NEORX) 20 September 1990, see the whole document ---	1-14
Y	J. Nucl. Med., vol. 31, no. 5, May 1990, (New York, US), L.C. KNIGHT et al.: "Thrombus imaging with TC-99M synthetic peptides reactive with activated platelets", page 757, abstract no. 209, see abstract (cited in the application) ---	1-14
P,Y	Chemical Abstracts, vol. 115, 1991, (Columbus, Ohio, US), B. LI et al.: "A new bifunctional chelating agent alpha,xi-N,N'-bis(L-cysteinyl)-L-lysine for radiolabeling of monoclonal antibodies with technetium-99M", see page 2001, abstract no. 159733f, & CHIN. CHEM. LETT. 1991, 2(4), 285-8, see abstract ---	1-14
A	Chemical Abstracts, vol. 114, 1991, (Columbus, Ohio, US), see page 850, abstract no. 186080b, & JP,A,02268197 (ICHIKAWA WOOLEN TEXTILE CO., LTD) 1 November 1990, see abstract ---	
A	EP,A,0108406 (F. HOFFMANN-LA ROCHE AND CO.) 16 May 1984, see claims (cited in the application) -----	

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: PLEASE SEE REMARK!!!!  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claim 12 is directed to a diagnostic method practised on the human/animal body the search has been carried out and based on the alleged effects of the compound / composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9200757  
SA 57367

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 21/07/92. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 9015818	27-12-90	EP-A- 0429626 GB-A- 2241243	05-06-91 28-08-91
WO-A- 9006323	14-06-90	None	
EP-A- 0284071	28-09-88	AU-B- 619738 AU-A- 1375188 JP-A- 1019058 US-A- 4965392 US-A- 5091514	06-02-92 29-09-88 23-01-89 23-10-90 25-02-92
EP-A- 0137457	17-04-85	US-A- 4732864 AU-B- 578428 AU-A- 3362984 CA-A- 1248090 DE-A- 3469536 JP-A- 1294700 JP-A- 60166625	22-03-88 27-10-88 18-04-85 03-01-89 07-04-88 28-11-89 29-08-85
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